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GlcN was administered alone or in combination with a CS-based gel by a weekly intra-articular injection for 3 weeks. ECM organization was assessed by histology.
Methods: Healthy and OA chondrocytes were isolated from the tibial plateaus of human cadaveric articular cartilage, or from patients undergoing knee arthroplasty (NDRI, Philadelphia, USA). Cells were cultured in chondrocyte medium (CM) for 2 passages, then photopolymerized into 10% w/v PEGDA or 15% w/v PEGDA-RGD modified hydrogels. Constructs were cultured for 1, 7, and 28 days in CM supplemented with 0, 0.1, 1, and 2 mM GlcN, or in chondrogenic medium (CM+) with 2 mM GlcN. Cell viability was assessed by live-dead and DNA assays, while tissue formation by histology, GAG, and collagen assays. Gene analysis was performed by PCR. For in vivo studies, OA was induced in rats by mechanical destabilization of the medial meniscus. Articular cartilage changes were evaluated after 4 and 8 weeks to validate the model. Two mM GlcN solutions in phosphate buffer saline (PBS) or in CSMA-aldehyde gels were intra-articularly injected weekly for 3 weeks. PBS was injected in control joints. Aggrecan synthesis was chosen as a parameter to evaluate ECM regeneration and assessed by histology.
Results: OA cells were more susceptible to hydrogel encapsulation and culture than healthy chondrocytes, as shown by viability data. RGD motifs decreased cell mortality. Although donor dependent, histology and GAG assays showed trends of increasing ECM formation with increasing GlcN concentration. CM+ revealed a further chondrogenic enhancement as compared to CM. PCR confirmed the same trend for ECM related markers. The inflammatory response (Il-1β and TNF-α) of OA cells encapsulated in PEGDA decreased with increasing culturing time, while it increased with increasing GlcN concentration. Conversely, the anti-inflammatory response (Il-4) increased with increasing culture time and GlcN concentration. RGD motifs induced an upregulation of Il-1β and Il-4 over time and a downregulation with increasing GlcN concentration. TNF-α did not significantly change. Similar conclusions could be drawn for healthy chondrocytes, with the exception of an upregulation of Il-4 with increasing GlcN concentration.
From histological analysis, GlcN alone or in combination with CSMA-aldehyde gel induced a significant slow down of arthritic damage and initial repair in rats that developed mild OA. Partial or minimal slow down of OA and repair was induced at a late OA stage.
Conclusions: GlcN administration to OA chondrocytes enhanced cellular ECM formation and modulated the inflammatory and anti-inflammatory responses in vitro. GlcN and GlcN-CSMA administrations initiated cartilage regeneration in OA rats in vivo. GlcN could be a potential factor for mild OA treatments and for the regeneration of cartilage in tissue engineering applications.

481 IN-ADVANCE TRANS-MEDULLARY STIMULATION OF MESENCHYMAL STEM CELLS ENHANCES SPONTANEOUS REPAIR OF FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS

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Purpose: Mesenchymal stem cells (MSCs) have the potential to differentiate into cartilage, suggesting that the MSCs, especially those close to the cartilage defects, are an important cell source for cartilage regeneration. In the present study, we stimulated MSCs to differentiate into the chondorcyte progenitors *via* trans-medullary approach using the rabbit knees. Following the trans-medullary stimulation of MSCs, full-thickness cartilage defects were created in order to investigate whether the chondorcyte progenitors stimulated thereby could enhance the repair of the full-thickness cartilage defects.
Methods: The animals were divided into 3 groups: In Group 1, full-thickness articular cartilage defects (5 mm in diameters; 5 mm in depth) were created in the patella groove of the femur. This group served as the negative control. In Group 2, trans-medullary holes (2.5 mm in diameter) reaching to the immediate vicinity of the subchondral bone were created 4 days prior to the creation of cartilage defects. In Group 3, bFGF was administered through the trans-medullary holes in order to augment the stimulation of the MSCs. Other procedures followed those of the group 2. The rabbits were sacrificed at 1, 2, 3, 4 and 8 weeks after the creation of the osteochondral defects, and Bromodeoxyuridine (BrdU) positive cells were enumerated and the histological grading score was counted.
Results: Enumerated BrdU positive cells indicated proliferation of the cells was significantly enhanced in Group 2 and 3 as compared with in that of Group 1. The histological grading score of in Group 3 was superior to the other groups and the score of the Group 1 remained the worst throughout the studied periods.
Conclusions: We demonstrated that the in-advance stimulation of MSCs *via* trans-medullary approach effectively increased the population of chondrocyte progenitors. The chondorcyte progenitors stimulated thereby are likely to be recruited to the osteochondral defects at the appropriate time, contributing to the repair of full-thickness articular cartilage defects.

482 ENDOCHONDRAL OSSIFICATION: AN ALTERNATIVE APPROACH FOR BONE REPAIR?

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Purpose: Bioengineered cell constructs can be used in the treatment of bone defects. Often Bone Marrow Stromal Cells (BMSCs) are used that are in-vitro stimulated towards the osteogenic lineage aiming at intramembranous bone formation. The success of this approach is disappointing. A major concern in these constructs is core degradation, which is caused by a lack of blood supply. We hypothesize that stimulation of cells towards the endochondral ossification process would be more successful. In this study we test how in-vitro priming of human BMSCs influences the endochondral ossification process.
Methods: *In vivo implantation*: hBMSCs were seeded in collagen-GAG scaffolds (3.0×10^5 per 8 mm^2). Seeded scaffolds were cultured for 21 days on chondrogenic medium (n = 5) (DMEM-LG, TGF-beta2 10 ng/ml, Dexamethasone (Dex) 10^{-7} M, Ascorbic acid 25 μg/ml, ITS) or osteogenic medium (n = 4) (DMEM-HG, 10% FCS, Dex 10^{-7} M, Beta-Glycerol-Phosphate (BGP) 10 mM, Ascorbic acid 0.1 mM) before they were subcutaneously implanted at the back in nude mice. After 28 days the scaffolds were obtained. Immunohistochemistry for Col-2 and Col-10 and Von Kossa/Thionin staining, to observe mineralization and glycosaminoglycan production, were performed.
In vitro pellet cultures: hBMSCs of three donors were obtained during hip arthroplasty. 2.0 10^5 cells were cultured in pellets in 0.5 ml of chondrogenic medium (as above). After 21 days of culture, when cartilage was formed, the cultures were divided in three groups to be cultured for another 14 days: group1 remained on chondrogenic medium; group 2 remained on chondrogenic medium and had BGP (10 mM) added to allow mineralization; in group 3 TGF-beta was removed from the medium, BGP (10 mM) was added and Dex was reduced to 10^{-8} M (osteogenic medium). At day 7, 14, 21, 35 immunohistochemistry for Col-2 and Col-10 and Von Kossa/Thionin staining were performed. On the culture medium of day 14, 21, 35 VEGFa production was determined by ELISA and gelatin zymography for MMP-production was performed. For each analysis 3 pellets were used. All outcome measures were statistically evaluated using mixed model analysis.
Results: *In vivo implantation*: Scaffolds that were pre-cultured on chondrogenic medium showed Col-2 and Col-10 production, but no mineralization. Moreover, vessels ingrowth that contained erythrocytes were present. Pre-culturing with osteogenic medium induced mineralization, but pycnotic cell nuclei were present and no Col-10 or vessel ingrowth were observed.
In vitro pellet cultures: Pellets cultured in chondrogenic medium showed progressive production of Col-2 and Col-10. In the medium of these chondrogenic cultured pellets VEGF and MMPs were evidently present at day 14, 21, 35. When pellets were switched to culture medium containing BGP, independent of the presence of TGF-beta, huge regions of mineralization were found. In the medium of these switched conditions VEGF and MMP productions had severely diminished.
Conclusions: We demonstrated that *in vivo* implantation of *in vitro* chondrogenically primed hBMSCs results in vessel invasion and Col-10 production, but not mineralization. By showing that VEGF and MMPs were produced in chondrogenically differentiated hBMSCs we demonstrated that these cells produce anabolic and catabolic factors that are known to be important for the conversion of an avascularized to a vascularized matrix. Inducing mineralization in this endochondral process does, however severely diminish these capacities. Altogether these results suggest that chondrogenical priming of hBMSCs may improve vessel invasion in bioengineered constructs, thereby optimizing its capacity to repair bone defects.